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Reviewer: Keisha Douglas

Timestamp: [year=2008; month=6; day=12; hr=19; min=27; sec=32; ms=818;]

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Application No: 10566827

Version No: 1.1

Input Set:

Output Set:

Started: 2008-06-12 19:25:59.156

Finished: 2008-06-12 19:26:01.131

Elapsed: 0 hr(s) 0 min(s) 1 sec(s) 975 ms

Total Warnings: 20

Total Errors: 0

No. of SeqIDs Defined: 22

Actual SeqID Count: 22

Error code	Error Description
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W 213	Artificial or Unknown found in <213> in SEQ ID (21)
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Input Set:

Output Set:

Started: 2008-06-12 19:25:59.156
Finished: 2008-06-12 19:26:01.131
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Total Errors: 0
No. of SeqIDs Defined: 22
Actual SeqID Count: 22

Error code

Error Description

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SEQUENCE LISTING

<110> de Lorenzo Prieto, Victor
Fernandez Herrero, Luis A

<120> System for the Production of Dimeric Proteins Based on the
Transport System of Hemolysin of Escherichia Coli

<130> 020884-000001

<140> 10/566,827

<141> 2006-01-31

<150> P200301830 (ES)

<151> 2003-07-31

<150> PCT/ES2004/070053

<151> 2004-07-19

<160> 22

<170> PatentIn version 3.4

<210> 1

<211> 36

<212> PRT

<213> Artificial

<220>

<223> EHlyA polypeptide containing 23 kDa ('hlyA) secretion signal of
E. coli Hly transporter tagged with the E epitope.

<400> 1

Met Thr Met Ile Thr Asn Leu Asp Leu Asn Ser Val Ser Thr Pro Gly
1 5 10 15

Gly Ala Pro Val Pro Tyr Pro Asp Pro Leu Glu Pro Ala Gly Glu Asn
20 25 30

Ser Leu Ala Lys
35

<210> 2

<211> 74

<212> PRT

<213> Artificial

<220>

<223> ZEHlyA polypeptide containing the 23 kDa ('hlyA) secretion signal
of E. coli Hly transporter tagged with the E epitope.

<400> 2

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 1 5 10 15

Gly Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Arg Met Lys Leu Glu
 20 25 30

Asp Lys Val Glu Glu Leu Leu Ser Lys Asn Tyr His Leu Glu Asn Glu
 35 40 45

Val Ala Arg Leu Lys Lys Leu Val Gly Glu Arg Gly Gly His His His
 50 55 60

His His His Ser Thr Pro Gly Gly Ala Pro
 65 70

<210> 3
 <211> 949
 <212> DNA
 <213> Artificial

<220>
 <223> Ampicillin resistant plasmid pZEHlyA (sense strand); 23-kDa
 C-terminal domain of HlyA with E-tag epitope incorporated at the
 23-kDa C domain of HlyA

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 acagctggaa gacaaagtag aggagctcct tagcaagaac taccatctag aaaacgaggt 180
 agctcgtctg aaaaagcttg ttggtgaacg tgggtggcac catcaccatc accatgcgtc 240
 gacgcccggg ggtgcgccgg tgccgtatcc ggatccgctg gaaccggccg gggaaaattc 300
 tcttgctaaa aatgtattat ccggtggaaa aggtaatgac aagttgtacg gcagtgaggg 360
 agcagacctg cttgatggcg gagaagggaa tgatcttctg aaaggtggat atggtaatga 420
 tatttatcgt tatctttcag gatatggcca tcatattatt gacgatgaag gggggaaaga 480
 cgataaactc agtttagctg atatagattt ccgggacggt gcctttaagc gagaagggaa 540
 tgacctcatt atgtataaag ctgaaggtaa tgttctttct attggccaca aaaatggat 600
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 tcagcagagt aataacaagg taagttatgt gtatggacat gatgcatcaa cttatgggag 780

ccaggacaat cttaatccat taattaatga aatcagcaaa atcatttcag ctgcaggtaa 840
 cttcgaatgtt aaggaggaaa gatctgccgc ttctttattg cagttgtccg gtaatgccag 900
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<210> 4
 <211> 918
 <212> DNA
 <213> Artificial

<220>
 <223> Ampicillin resistant plasmid pZEHlyA (missense strand); 23-kDa
 C-terminal domain of HlyA with E-tag epitope incorporated at the
 23-kDa domain of HlyA.

<400> 4
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 tcgttcttga tggtagatct tttgtccat cgagcagact ttttcgaaca accacttgca 180
 ccaccagtgg tagtggtagt ggtacgcagc tgcgggcccc cacgcggcca cggcataggc 240
 ctaggcgacc ttggccggcc ccttttaaga gaacgatttt tacataatag gccacctttt 300
 ccattactgt tcaacatgcc gtcactccct cgtctggacg aactaccgcc tcttccctta 360
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 gtataataac tgctacttcc cccctttctg ctatttgagt caaatcgact atatctaaag 480
 gccttgcaac ggaaattcgc tcttccctta ctggagtaat acatatttcg acttccatta 540
 caagaaagat aaccggtgtt ttaccataa tgtaaatttt tgaccaaact ttttctcagt 600
 ctactagaga gattagtagt ctatctcgtc taaaaactat ttctgccgtc ccattagtgt 660
 ggtctaagag aatttttttcg taaacttata gtcgtctcat tattgttcca ttcaatacac 720
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<210> 5
 <211> 305
 <212> PRT
 <213> Artificial

<220>

<223> Ampicillin resistant plasmid pZEHlyA (protein); 23-kDa C-terminal domain of HlyA with E-tag epitope incorporated at the 23-kDa domain of HlyA.

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1 5 10 15

Gly Pro Lys Pro Ser Thr Pro Pro Gly Ser Ser Arg Met Lys Gln Leu
20 25 30

Glu Asp Lys Val Glu Glu Leu Leu Ser Lys Asn Tyr His Leu Glu Asn
35 40 45

Glu Val Ala Arg Leu Lys Lys Leu Val Gly Glu Arg Gly Gly His His
50 55 60

His His His His Ala Ser Thr Pro Gly Gly Ala Pro Val Pro Tyr Pro
65 70 75 80

Asp Pro Leu Glu Pro Ala Gly Glu Asn Ser Leu Ala Lys Asn Val Leu
85 90 95

Ser Gly Gly Lys Gly Asn Asp Lys Leu Tyr Gly Ser Glu Gly Ala Asp
100 105 110

Leu Leu Asp Gly Gly Glu Gly Asn Asp Leu Leu Lys Gly Gly Tyr Gly
115 120 125

Asn Asp Ile Tyr Arg Tyr Leu Ser Gly Tyr Gly His His Ile Ile Asp
130 135 140

Asp Glu Gly Gly Lys Asp Asp Lys Leu Ser Leu Ala Asp Ile Asp Phe
145 150 155 160

Arg Asp Val Ala Phe Lys Arg Glu Gly Asn Asp Leu Ile Met Tyr Lys
165 170 175

Ala Glu Gly Asn Val Leu Ser Ile Gly His Lys Asn Gly Ile Thr Phe
180 185 190

Lys Asn Trp Phe Glu Lys Glu Ser Asp Asp Leu Ser Asn His Gln Ile
195 200 205

Glu Gln Ile Phe Asp Lys Asp Gly Arg Val Ile Thr Pro Asp Ser Leu
210 215 220

Lys Lys Ala Phe Glu Tyr Gln Gln Ser Asn Asn Lys Val Ser Tyr Val
225 230 235 240

Tyr Gly His Asp Ala Ser Thr Tyr Gly Ser Gln Asp Asn Leu Asn Pro
245 250 255

Leu Ile Asn Glu Ile Ser Lys Ile Ile Ser Ala Ala Gly Asn Phe Asp
260 265 270

Val Lys Glu Glu Arg Ser Ala Ala Ser Leu Leu Gln Leu Ser Gly Asn
275 280 285

Ala Ser Asp Phe Ser Tyr Gly Arg Asn Ser Ile Thr Leu Thr Ala Ser
290 295 300

Ala
305

<210> 6
<211> 1979
<212> DNA
<213> Artificial

<220>

<223> Ampicillin resistant plasmid pZEHLA2SD (sense strand); 23-kDa
C-terminal domain of HlyA with E-tag epitope incorporated at the
23-kDa domain of HlyA and polylinker for cloning of scFv's in
frame with E-tagged 'hlyA.

<400> 6
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gagaccacaa cgggtttccct ctagaaataa ttttggttaa cttaagaag gagatatatc 120
catggctagc acggcctcgg gggccgcgtc gacgtccggc ggtccgaagc cttccactcc 180
gcccggttct tcccgtatga aacagctgga agacaaagta gaggagctcc ttagcaagaa 240
ctaccatcta gaaaacgagg tagctcgtct gaaaaagctt gttggtgaac gtggtggtca 300
ccatcaccat caccatgcgt cgacgcccg ggtgcgccg gtgccgtatc cggatccgct 360
ggaaccggcc ggggaaaatt ctcttgctaa aaatgtatta tccggtggaa aaggtaatga 420
caagttgtac ggcagtgagg gacgagacct gcttgatggc ggagaaggga atgatcttct 480

gaaaggtgga tatggtaatg atatttatcg ttatctttca ggatatggcc atcatattat	540
tgacgatgaa ggggggaaag acgataaact cagtttagct gatatagatt tccgggacgt	600
tgcctttaag cgagaaggga atgacctcat tatgtataaa gctgaaggta atgttctttc	660
tattggccac aaaaatggta ttacatttaa aaactggttt gaaaaagagt cagatgatct	720
ctctaatacat cagatagagc agatTTTTga taaagacggc agggtaatca caccagattc	780
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aatcatttca gctgcaggta acttcgatgt taaggaggaa agatctgccg cttctttatt	960
gcagttgtcc ggtaatgcc a gtgatttttc atatggacgg aactcaataa ctttgacagc	1020
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gctatTTTT tggagtcata atggattctt gtcataaaat tgattatggg ttatacgccc	1140
tggagatttt agcccaatac cataacgtct ctgttaacct ggaagaaatt aaacatagat	1200
ttgacacaga cgggactggc ctgggattaa cgtcatgggt gcttgcgcg aaatctttag	1260
aactaaaggt aaaacaggta aaaaaaaca ttgaccgatt aaactttatt tctctgcccg	1320
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acagatatct tatttctgat ctggagcagc gaaatccccg tgttctcgaa cagtctgagt	1440
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aactggcgaa atttgacttt acctggttta ttcctgccat tataaaatac aggagaatat	1560
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<210> 7

<211> 1979

<212> DNA

<213> Artificial

<220>

<223> Ampicillin resistant plasmid pZEHLA2SD (missense strand); 23-kDa C-terminal domain of HlyA with E-tag epitope incorporated at the 23-kDa domain of HlyA and polylinker for cloning of scFv's in frame with E-tagged 'hlyA.

<400> 7

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gtaccgatcg tgccggagcc cccggcgagc ctgcaggccg ccaggcttcg gaaggtgagg	180
cgggccca gaagggcact ttgtcgacct tctgtttcat ctctcgagg aatcgttctt	240
gatggtagat cttttgctcc atcgagcaga ctttttcgaa caaccacttg caccaccagt	300
ggtagtggtg gtggtacgca gctgcggggc cccacgcggc cagggcatag gcctaggcga	360
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ctttccacct ataccattac tataaatagc aatagaaagt cctataccgg tagtataata	540
actgctactt ccccccttct tgcattttga gtcaaatcga ctatatctaa aggccttgca	600
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ataaccggtg tttttaccat aatgtaaatt ttgacccaa ctttttctca gtctactaga	720
gagattagta gtctatctcg tctaaaaact atttctgcgg tcccattagt gtggtctaag	780
agaatttttt cgtaaaacta tagtcgtctc attattgttc cattcaatac acatacctgt	840
actacgtagt tgaataccct cggctcgtgt agaattaggt aattaattac tttagtcgtt	900
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acctctaaaa tcgggttatg gtattgcaga gacaattggg ccttctttta tttgtatcta	1200
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aaaaagtcca ccaatacctg tttcataatc acgtgtcccc taaaagttga gaattacaat 1680
aatgacagcg taatagacaa caccaccaca aactctaata tgagtcgcca aattcttgaa 1740
tgtaaaaacg tgtatcatgt tcagcctaac tacaactcaa cccacggttt gagaaggccg 1800
taaattgaccg cgatggctag agaataaaac tctcagcagc acaaccacta tgacaacggt 1860
cccatctctt taatctgggc taggcattaa aagactgtcc tgtccgtaat tgtagacaag 1920
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<210> 8

<211> 302

<212> PRT

<213> Artificial

<220>

<223> Ampicillin resistant plasmid pZEHLA2SD (protein); 23-kDa
C-terminal domain of HlyA with E-tag epitope incorporated at the
23-kDa domain of HlyA and polylinkerfor cloning of scFv's in
frame with E-tagged 'hlyA.

<400> 8

Met Ala Ser Thr Ala Ser Gly Ala Ala Ser Thr Ser Gly Gly Pro Lys
1 5 10 15

Pro Ser Thr Pro Pro Gly Ser Ser Arg Met Lys Gln Leu Glu Asp Lys
20 25 30

Val Glu Glu Leu Leu Ser Lys Asn Tyr His Leu Glu Asn Glu Val Ala
35 40 45

Arg Leu Lys Lys Leu Val Gly Glu Arg Gly Gly His His His His His
50 55 60

His Ala Ser Thr Pro Gly Gly Ala Pro Val Pro Tyr Pro Asp Pro Leu
65 70 75 80

Glu Pro Ala Gly Glu Asn Ser Leu Ala Lys Asn Val Leu Ser Gly Gly
85 90 95

Lys Gly Asn Asp Lys Leu Tyr Gly Ser Glu Gly Ala Asp Leu Leu Asp
100 105 110

Gly Gly Glu Gly Asn Asp Leu Leu Lys Gly Gly Tyr Gly Asn Asp Ile
115 120 125

Tyr Arg Tyr Leu Ser Gly Tyr Gly His His Ile Ile Asp Asp Glu Gly
130 135 140

Gly Lys Asp Asp Lys Leu Ser Leu Ala Asp Ile Asp Phe Arg Asp Val
145 150 155 160

Ala Phe Lys Arg Glu Gly Asn Asp Leu Ile Met Tyr Lys Ala Glu Gly
165 170 175

Asn Val Leu Ser Ile Gly His Lys Asn Gly Ile Thr Phe Lys Asn Trp
180 185 190

Phe Glu Lys Glu Ser Asp Asp Leu Ser Asn His Gln Ile Glu Gln Ile
195 200 205

Phe Asp Lys Asp Gly Arg Val Ile Thr Pro Asp Ser Leu Lys Lys Ala
210 215 220

Phe Glu Tyr Gln Gln Ser Asn Asn Lys Val Ser Tyr Val Tyr Gly His
225 230 235 240

Asp Ala Ser Thr Tyr Gly Ser Gln Asp Asn Leu Asn Pro Leu Ile Asn
245 250 255

Glu Ile Ser Lys Ile Ile Ser Ala Ala Gly Asn Phe Asp Val Lys Glu
260 265 270

Glu Arg Ser Ala Ala Ser Leu Leu Gln Leu Ser Gly Asn Ala Ser Asp
275 280 285

Phe Ser Tyr Gly Arg Asn Ser Ile Thr Leu Thr Ala Ser Ala
290 295 300

<210> 9

<211> 2792

<212> DNA

<213> Artificial

<220>

<223> Ampicillin resistant plasmid pVamyHLYA (sense strand) containing
amplified DNA product encoding VHH amylase (Vamy); 23-kDa
C-terminal domain of HlyA with E-tag epitope incorporated at the

23-kDa domain of HlyA and polylinker for cloning of scFv's in

<400> 9

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tggtaccgcc aggctgcagg gaagcagcgc gagtgggtct catctattag tactgatggt	240
cgcacaagct atgcagactc cgtgaagggc cgattcacca tctccaaaga caaagccaag	300
gacacgggtgt atctgcaa atgaacagcctg aaacctgagg acacggccat ctattactgt	360
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gaaaaagagt cagatgatct ctctaatacat cagatagagc agatttttga taaagacggc	900
agggtaatca caccagattc tcttaaaaaa gcatttgaat atcagcagag taataacaag	960
gtaagttatg tgtatggaca tgatgcatca acttatggga gccaggacaa tcttaatcca	1020
ttaattaatg aaatcagcaa aatcatttca gctgcag	